Journal of Genetic Engineering and Biotechnology (2012) **10**, 87–92

Academy of Scientific Research & Technology and
National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ARTICLE

Renal leptin in experimental nephrotic syndrome

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Received 20 September 2011; revised 30 November 2011; accepted 11 December 2011
Available online 10 February 2012

KEYWORDS

Leptin;
Nephrotic syndrome;
Lipids;
Lipoproteins;
Apolipoproteins;
Rats

Abstract Leptin is a fat derived hormone involved in the regulation of metabolism and body composition. The kidney is the principle organ responsible for elimination of circulating leptin. Our aim is to evaluate if the nephrotic kidneys participate in the metabolism of leptin by comparing the serum leptin level in renal veins and in their renal arteries and to study the relationship between leptin and lipoprotein levels in healthy and nephrotic rats. *Methods*: Rats were divided into two equal groups: group 1 in which experimental nephrotic syndrome was produced by injecting them intraperitoneally with a supernatant of the homogenized mixture of their own kidney (obtained by previous unilateral nephrectomy) and complete Freund's adjuvant. Another group constituted the control group. Leptin and lipid profile were estimated in blood samples of renal veins and renal arteries. There was a highly significant increase in leptin and lipid profile levels in the nephrotic rats compared with the normal group. There was a high significant decrease in leptin in the renal venous blood compared with its level in the renal arterial blood of normal and nephrotic rats. This work has stressed the involvement of kidney and the nephrotic renal tissue in the process of leptin metabolism and lipogenesis.

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Peer review under National Research Center, Egypt.
doi:10.1016/j.jgeb.2011.12.004

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1. Introduction

Leptin (from the Greek word leptos, meaning thin) was firstly identified in 1994. It is the product of ob gene [46]. It is a fat derived hormone involved in the regulation of metabolism and body composition [3]. Wasilewska et al. [43] demonstrated that the kidneys play a key role in the systemic elimination of circulating leptin. Leptin is not metabolized by the kidney but is excreted as an intact protein. The mechanism involved is a high capacity non-saturable process, most probably glomeru-

lar filtration [4]. Nephrotic syndrome (NS) is a well recognized renal disorder of varied etiology; and is characterized by a special plasma lipids and lipoproteins profile. Impaired renal function is found in nephrotic syndrome. Landt et al. [24] found a relationship between impaired renal function and abnormal leptin metabolism [20].

Leptin concentrations are increased in the plasma of patients with end-stage renal disease and chronic renal failure [27]. An increased protein filtration in nephrotic syndrome increases urinary leptin excretion. However, the increased leptin loss in the urine is not always accompanied by a decrease in serum levels[40]. Kovesdy et al. [23] found elevated leptin level in kidney transplant recipients.

Wasilewska et al. [43] found increased urinary leptin excretion in patients with nephrotic syndrome accompanied by normal serum levels. Another important mechanism which may be involved in this process is the role of soluble leptin receptor in serum of nephrotic patients. It was found that levels of soluble leptin receptor, identified as leptin binding protein, were significantly higher in nephrotic patients than in controls and patients in remission [4]. Other investigators had observed an increase in urinary leptin excretion concomitant with a reduction in the serum level [28]. In nephrotic syndrome children leptin urinary excretion increases but its level in serum is unchanged. Serum leptin level is correlated with lipid parameters [43].

Ece et al. [10] demonstrated that plasma leptin levels in (NS) remain controversial. Leptin structurally resembles a cytokine and a number of animal studies suggest a link between inflammation and elevated leptin levels [34,13]. Leptin's role in the regulation of lipid metabolism has not been completely elucidated [16,44,38].

Wu et al. [44] showed in healthy children a positive correlation of serum leptin level with triglycerides (TG), intermediate-density lipoprotein (IDL) and apolipoprotein B (apo B), and a negative correlation with high-density lipoprotein (HDL) and apolipoprotein A (apo A). Other authors showed a positive correlation between leptin, triglycerides (TG), apolipoprotein B (apo B) [33,44,43].

Dashti et al. [8] concluded that the kidney contributes to clearance of circulating leptin in humans and further studies are required to evaluate the significance of these elevated leptin levels in relation to indices of appetite in patients with end-stage renal disease. Future research should also focus on elucidating the etiology of the inflammation and studying the long-term effects of various anti-inflammatory treatment strategies on nutritional and cardiovascular status as well as outcome in end-stage renal disease (ESRD) patients.

Many workers suggested that liver is the source of the elevated plasma lipids in nephrotic syndrome [25,2]. Nicola et al. [30,31], suggested that nephrotic kidney is a source of elevated lipids profile in view of the significantly increased lipids profile level in the nephrotic renal venous blood than in the nephrotic renal arterial blood.

The aim of this work is to evaluate if the nephrotic kidneys participate in the metabolism of leptin by comparing the serum leptin level in renal veins with that in the renal arteries of rats with nephritic syndrome and to study the relationship between leptin and lipoprotein levels in healthy and nephrotic rats.

2. Materials and methods

Thirty male albino rats weighed from 160 to 190 g, taken from the animal house of the National Research Centre, were used. The rats were fed on a stock diet composed of whole wheat meal, skimmed milk powder, fish flour, dry yeast, stabilized vitamin A, D and NDP energy of 12% [29]. Diet was given in plenty to the rats throughout the experimentation period (for 3 months) Animals were provided with water *ad-libitum*. The animal experiments were done in accordance with the regulations of the Ethical Committee of the National Research Centre.

2.1. Study design

The rats were divided into two equal groups (each group: 15 rats): Group I: Control group I, 15 healthy male rats served as healthy controls. The other group (Group II) had their kidneys made nephrotic by injecting them intraperitoneally with 0.2 cc low speed supernatant (3000–4000 rpm for 1–1.5 h) of 1:2 blood free own kidney in saline obtained by previous unilateral nephrectomy with 0.3 Freund's complete adjuvant (is an antigen emulsified in mineral oil, used to stimulate production of tumor necrosis factor). Blood samples of their levels in their renal veins with those in their venous blood and also with those of normal control rats. Each rat was injected whole six injections one each 14 days) after the technique of [18].

2.2. Unilateral nephrectomy

General anesthesia of rats was done through inhalation of diethyl ether, after anesthesia, the hair at the site of operation was clipped and shaved then washed by soap and water, and disinfected by alcohol 70%, followed by Tincture iodine 4%. A longitudinal incision in the skin and muscles was made, and by blunt forceps the wound was widened till appearance of the kidney. Strong ligation to the renal artery and renal vein were made separately followed by excision of kidney. After nephrectomy, coaptation of the wound occurred by lemberet's sutures. Intraperitoneal injections of operated rats had been done after complete recovery for about four weeks.

Blood was collected from renal artery and renal vein by using syringe into dry clean test tubes, then allowed to clot and centrifuged at 4000 rpm for 10 min to separate the serum. Serum was collected into dry clean test tubes; glucose and low density lipoprotein (LDL-cholesterol) were determined immediately. The rest of serum was frozen at −20 °C for the subsequent estimation of the other parameters.

2.3. Estimation of serum leptin

Leptin was estimated by an immunoenzymometric assay for the quantitative measurement of human leptin in serum and plasma "The Biosource Leptin Enzyme Amplified Sensitivity Immuno assay (EASIA)". The kit was supplied by BioSource Europe SA, rue de l' Industrie 8 B-1400 Nivelles Belgium.

2.4. Determination of serum total cholesterol

Total cholesterol was determined using the enzymatic method of Allain et al. [1]. Using the total cholesterol kit of Bio-Merieux

laboratory reagents and products. Bio-Merieux 69280 Marcy: L'Etoile/France.

2.5. Determination of serum triglycerides

Serum triglycerides were determined by the quantitative enzymatic calorimetric determination of triglycerides according to Wahlefeld [42] by using Stanbio Enzymatic, 2930 East Houston Street, San Antonio, Texas 78202, USA.

2.6. Determination of serum phospholipids

Serum phospholipid was determined by enzymatic method according to Trinder [39] using phospholipids kit of Quimica Clinica Aplicada 43870 Amposta/Tarragona. Espana.

2.7. Determination of serum HDL-cholesterol

Was done according to Glatter equation, Glatter [14] where:

$$\text{Serum HDL-Cholesterol} = \text{Total cholesterol} - \frac{\text{Triglyceride}}{5} - \text{LDL-Cholesterol}$$

2.8. Determination of serum LDL-cholesterol

Serum LDL cholesterol was determined after Steinberg [37] by using LDL cholesterol/phospholipids kit of Bio Merieux laboratory reagents and products Bio Merieux 69280 Marcy-1, Etoile/France.

2.9. Determination of serum VLDL-cholesterol

According to Glatter equation, Glatter [14] where

$$\text{Serum VLDL Cholesterol} = \frac{\text{Triglycerides}}{5}$$

2.10. Determination of serum apolipoprotein A-1 and B

Apolipoprotein A-1 and B were estimated by immunoprecipitin analysis method according to Finley et al. [11]. Apo A-1 kits obtained from Atlantic Antibodies, An INCSTAR Company INCSTAR corporation-Stillwater, Minnesota, USA.

2.11. Statistical analysis of data

Data are expressed as mean ± SE. Statistical significance of the difference was analyzed using one way-ANOVA and post-hoc Duncans' test for multiple group comparison. P values of < 0.05 were considered statistically significant. The correlation coefficient (r) which is a measure of the degree of closeness of the linear relationship between two variables (X and Y) was determined, r always lies between -1 and $+1$.

3. Results

There was a highly significant decrease in leptin level in renal veins compared with its level in renal arteries of normal rats. There was also a non significant rise in apolipoprotein A-1 and apolipoprotein B value in renal veins compared with the values in renal arteries blood of normal rats. High significant decrease in leptin level was seen in renal veins compared with renal arteries of nephrotic rats. In addition, we observed a highly significant increase in apolipoprotein A-1 and apolipoprotein B in renal veins compared with the renal arteries of nephrotic rats (Table 1). There was also a high serum leptin, apolipoprotein A-1 and apolipoprotein B levels in the nephrotic rats compared with the normal controls (Table 1). There was an insignificant change in lipid profile between the renal vein compared with renal artery blood of normal rats (Table 2), but a significant rise in lipid profile in the renal vein compared with its level in renal artery of nephrotic rats (Table 2). There was also a high serum lipid profile level in the nephrotic rats compared with the normal controls (Table 2). Table 3 shows the correlation between renal arterial serum leptin and lipids in normal control and nephrotic rats. Table 4 shows the correlation between renal venous serum leptin and lipids in normal control and nephrotic rats.

4. Discussion

In the present work, there is a high significant decrease in leptin in the renal venous blood than in the renal arterial blood of normal rats (Table 1). This is mainly because the kidneys play a principal role in the elimination of this peptide [36]. The kidney is the principle organ responsible for elimination of circulating leptin. Arteriovenous balance studies in rodents and humans showed that the kidney efficiently extracted leptin from the renal circulation [24]. Many authors demonstrated that endogenous leptin is rapidly cleared from the circulation by the kidney by glomerular filtration followed by metabolic degradation in the renal tubules [43,35,6]. However few authors reported that leptin is not metabolized by the kidney but is excreted as an intact protein. The mechanism involved is a high capacity non-saturable process, most probably glomerular filtration [4].

In the present work, there was a high significant decrease in leptin in the renal venous blood than in the renal arterial blood of nephrotic rats (Table 1). This is due to increasing of leptin urinary excretion and which agrees with Wasilewska et al. [43] who demonstrated that in nephrotic children; leptin urinary excretion increases. It was also suggested that the significant loss of leptin in proteinuric patients is compensated by sustained upregulation of leptin production [35].

Impaired renal function is found in nephrotic syndrome and Landt et al. [24] found relationship between impaired renal function and abnormal leptin metabolism [4].

The cascade of events responsible for leptin catabolism by the kidneys is unknown. The failure of leptin to appear immediately in urine after bolus injection is inconsistent with the hypothesis that leptin is eliminated by simple glomerular filtration alone. The results of these kinetic studies suggest that renal metabolism of leptin could involve active uptake of leptin by renal tissues. The study of Cumin et al. [7] concluded that glomerular filtration was the important mechanism for renal elimination of leptin [45].

In the present work, there are high serum leptin levels in the nephrotic rats compared with the normal controls (Table 1).

Table 1 Serum leptin, apolipoprotein A-1 (Apo A-1) and apolipoprotein B (Apo B) levels in renal venous (RV) and renal arterial (RA) blood in different experimental groups.

Group (n = 9)	Leptin (ng/ml)	Apo A-1 (mg/dl)	Apo B (mg/dl)
RA-control	2.48 ± 0.057	47.68 ± 0.38	34.01 ± 0.41
RV-control	2.16 ± 0.033[a]	47.01 ± 0.26	34.46 ± 0.36
RA-nephrotic	3.68 ± 0.054[b]	103.57 ± 0.42[b]	79.98 ± 0.37[b]
RV-nephrotic	3.51 ± 0.048[c,d]	120.46 ± 0.54[c,d]	89.97 ± 1.02[c,d]

Statistical differences were observed between:
[a] $p < 0.05$, RA-control and RV-control.
[b] $p < 0.05$, RA-control and RA-nephrotic.
[c] $p < 0.05$, RA-nephrotic and RV-nephrotic.
[d] $p < 0.05$, RV-control and RV-nephrotic.

Table 2 Serum lipid fractions in renal venous (RV) and renal arterial (RA) blood in different experimental groups.

Group (n = 9)	TC (mg/dl)	TG (mg/dl)	Phosph (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
RA-control	80.16 ± 1.52	66.62 ± 0.63	132.87 ± 0.1	28.08 ± 1.56	38.76 ± 0.33	13.32 ± 0.13
RV-control	81.27 ± 1.38	66.68 ± 0.64	133.96 ± 0.9	28.80 ± 1.44	38.92 ± 0.27	13.34 ± 0.42
RA-nephrotic	135.38 ± 2.61[a]	153.71 ± 1.49[a]	201.80 ± 1.78[a]	58.64 ± 2.11[a]	45.99 ± 0.57[a]	30.74 ± 0.3[a]
RV-nephrotic	164.57 ± 2.01[b,c]	230.90 ± 2.16[b,c]	255.99 ± 1.64[b,c]	65.42 ± 2.39[b,c]	53.45 ± 0.48[b,c]	46.2 ± 1.4[b,c]

TC, total cholesterol; TG, triglycerides; Phosph, phospholipids; LDL, low-density lipoprotein.
HDL, high-density lipoprotein; VLDL, very low-density lipoprotein.
Statistical differences were observed between:
[a] p < 0.05, RA-control and RA-nephrotic.
[b] $p < 0.05$, RA-nephrotic and RV-nephrotic.
[c] $p < 0.05$, RV-control and RV-nephrotic.

Table 3 Correlation between renal arterial serum (RA) leptin and serum lipids in normal control and nephrotic rats.

Group		TC	TG	Phosph	HDL	LDL	VLDL	Apol A-1	Apo B
RA-control (n = 9)	r	0.403	0.928	−0.174	−0.244	0.980	0.075	−0.318	−0.214
	P	< 0.05	< 0.01	NS	NS	< 0.01	NS	NS	NS
RA-nephrotic (n = 8)	r	0.026	−0.164	0.081	0.095	0.044	−0.163	−0.186	−0.507
	P	NS	NS	NS	NS	NS	NS	NS	< 0.05

r, correlation coefficient; TC, total cholesterol; TG, triglycerides; Phosph, phospholipids; LDL, low-density lipoprotein, HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; APO A-1, apolipoprotein A-1; Apo B, apolipoprotein B.

Table 4 Correlation between renal venous (RV) serum leptin and serum lipids in normal control and nephrotic rats.

Group			TC	TG	Phosph	HDL	LDL	VLDL	Apol A-1	Apo B
RV-control	Leptin	r	−0.195	0.907	−0.182	−0.479	0.955	−0.0123	−0.387	−0.666
		P	NS	< 0.01	NS	< 0.05	< 0.01	NS	NS	< 0.05
RV-nephrotic	Leptin	r	0.283	0.428	0.337	0.453	0.345	0.428	0.142	−0.020
		P	NS	< 0.05	NS	< 0.05	NS	< 0.05	NS	NS

r, correlation coefficient; TC, total cholesterol; TG, triglycerides; Phosph, phospholipids; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; Apo A-1, apolipoprotein A-1; Apo B, apolipoprotein B.

Many authors demonstrated that no significant difference in plasma leptin concentrations between the healthy subjects and nephrotic patients [17,43,32].

However; Huang et al. [19] demonstrated that high levels of leptin can be caused by the delayed clearance of leptin from circulation due to the binding to its soluble receptor. They concluded that sOB-R is up-regulated and an over-expression of sOB-R results in an increase of circulating leptin in nephrotic syndrome [17]. These results are in accordance with the results of the present work. We add that our method of induction of nephrotic syndrome used unilateral nephrectomized rats so the amount of clearance and elimination of leptin by one kidney is less than that by two kidneys. This leads to increase of leptin in our finding.

Cohen et al. [5] indicated that the liver is a major source of plasma-soluble leptin receptor (SLR) expression in states of negative energy balance and demonstrate a novel role for the liver in modulating leptin action. The data showed that the relationship between leptin and lipid levels in patients with chronic glomerulonephritis is limited [26].

Wu et al. [44] demonstrated a positive correlation between serum leptin and triglyceride and LDL levels in healthy children, and a negative correlation between serum leptin and HDL; this coordinates with our finding in our control rats (Tables 3 and 4). Research suggested that there is a relationship between leptin concentration and certain markers of the metabolic syndrome, including cholesterol, triglycerides and apolipoproteins [12,44,38]. A substantial disturbance of lipid metabolism occurs in children with idiopathic nephrotic syndrome [9,41]. Haluzik et al. [15] concluded that serum leptin levels in most types of hyperlipidemia simply reflect the degree of adiposity expressed by BMI and are not clearly related to serum lipid and/or lipoprotein concentrations. Our results coordinate with Wasilewska et al. [43] who reported a positive correlation of leptin with total cholesterol, its LDL fraction and HDL fraction and a negative correlation with apo A and apo B in nephrotic children (Tables 3 and 4). However our results are not in agreement with them in correlation of leptin with triglyceride which a negative correlation in renal arterial blood and a positive correlation in renal venous of nephrotic rats (Tables 3 and 4).

Literature data referring to the relationship between serum leptin level and lipid fractions in nephrotic patients are scarce. Ozata et al. [32] found no correlation of leptin with plasma TG or other lipid parameters in nephrotic subjects. Thus, their data suggest that plasma leptin levels are normal in the nephrotic syndrome and do not play a role in the dyslipidemia observed in this syndrome.

Nephrotic syndrome is a well recognized renal disorder of varied etiology; and is characterized by a special plasma lipids and lipoproteins profile whatever the cause is. This lipids and lipoproteins profile shows hypercholesterolemia, raised LDL and VLDL pattern, and decreased HDL concentration [21]. The latter is especially manifest in human nephrotic syndrome [22].

This characteristic lipid profile in face of the varied etiology of the syndrome led Nicola et al. [30,31] to study the role of the kidney tissue in the genesis of plasma lipids in this characteristic pattern.

There is a highly important increase in serum lipids, lipoprotein fractions, apo A-1, apo B of nephrotic rats when compared with the control group, but the point is that, all these parameters were significantly increased in the renal venous blood of the nephrotic rats than in their renal arterial blood, a finding which was not seen in the control group. These results prove that kidney plays a role in lipogenesis in nephrosis.

References

[1] F.U.P.C., C.C. Allain, L.S. Poon, C.S.G. Chan, W. Richmond, Clin. Chem. 20 (1974) 470–475.

[2] D.B. Bernard, in: B.M. Brenner, J.H. Stein (Eds.), Contemporary Issues in Nephrology (vol. 9. Nephrotic Syndrome), Churchill Livingstone, New York, 1982, pp. 85–120.

[3] C. Buettner, E.D. Muse, A. Cheng, L. Chen, T. Scherer, A. Pocai, K. Su, B. Cheng, Nat. Med. 14 (2008) 667–675.

[4] N. Buyan, O. Ozakaya, A. Bideci, O. Soylemezo, P. Cinaz, S. Gonen, S. Kalman, S. Bakkalo, E. Hasano, Pediatr. Nephrol. 18 (2003) 1009–1014.

[5] Cohen Paul, Guoqing Yang, Yu Xinxin, Alexander. A. Soukas, Cara Wolfish, Jeffrey. M. Friedman, J. Biol. Chem. 280 (10034) (2005) 10034–10039.

[6] F. Cumin, H.P. Baum, N. Levens, J. Endocrinol. 155 (1997) 577–585.

[7] F. Cumin, H.P. Baum, N. Levens, Int. J. Obes. 20 (1996) 1120–1126.

[8] N. Dashti, E. Nahid, N. Fariba, Pak. J. Med. Sci. 24 (5) (2008) 694–697.

[9] E.E. Delvin, A. Merouani, E. Levy, Clin. Biochem. 36 (2003) 95–101.

[10] A. Ece, Y. Atamer, F. Gurkan, M. Bilici, Y. Kocyrigit, Nephrology 9 (6) (2004) 366–373.

[11] P.R. Finley, J.R. Williams, J.M. Bayers, Clin. Chem. 22 (7) (1976) 1037–1141.

[12] S. Fujimaki, T. Kanda, K. Fujta, J. Tamura, I. Kobayashi, J. Int. Med. Res. 29 (2001) 108–113.

[13] C.C. Gerhardt, I.A. Romero, R. Cancello, L. Camoin, A.D. Strosberg, Mol. Cell. Endocrinol. 7 (2001) 81–92.

[14] T.R. Glatter, Post-grad. Med. 76 (6) (1984) 49–55, 58–59.

[15] M. Haluzík, J. Fiedler, J. Nedvídková, R. Ceska, Nutrition 16 (6) (2000) 429–433.

[16] M. Haluzik, J. Fiedler, J. Nedvidkova, R. Ceska, Physiol. Res. 48 (1999) 363–368.

[17] Henmi. Kayo, Masaharu. Yoshida, Noriko. Yoshikawa, Iwao. Nakabayashi, Toshihiko. Hirano, Int. Immunopharmacol. 8 (2008) 1703–1706.

[18] W. Heymann, D.B. Hachel, S. Harwood, S.G.F. Wilson, J.L.P. Hunter, Proc. Soc. Exp. Biol. 100 (1959) 660–664.

[19] L. Huang, Z. Wang, C. Li, J. Biol. Chem. 276 (2001) 6343–6349.

[20] R.P. Hull, J.A. Goldsmith, BMJ 336 (7654) (2008) 1185–1189.

[21] J. Joven, C. Villabona, E. Vilella, L. Masana, R. Alberti, M. Valles, N. Engl. J. Med. 323 (1990) 579–584.

[22] G.A. Kaysen, R.W. Davies, G.A. Kaysen, Kidney Int. 39 (Suppl. 31) (1991) s8–s15.

[23] C.P. Kovesdy, M.Z. Molnar, M.E. Czira, A. Rudas, A. Ujszaszi, L. Rosivall, M. Szathmari, A. Covic, A. Keszei, G. Beko, P. Lakatos, J. Kosa, I. Mucsi, Clin. J. Am. Soc. Nephrol. 5 (12) (2010) 2297–2304.

[24] M. Landt, D. Brennan, C. Parvin, K. Flavin, S. Dagogo-Jack, D. Coyne, Nephrol. Dial. Transplant. 13 (9) (1998) 2271–2275, 5.

[25] J.B. Marsh, D.L. Drabkin, J. Biol. Chem. 230 (2) (1958) 1073–1081.

[26] A. Medyńska, K.P. Katarzyna, A. Zwolińska, A. Chorążyczewski, Adv. Clin. Exp. Med. 17 (2) (2008) 137–140, ISSN 1230-025X.

[27] E. Merabet, S. Dagogo-Jack, D.W. Coyne, S. Klein, J.V. Santiago, S.P. Hmiel, M. Landt, J. Clin. Endocrinol. Metab. 82 (1997) 847–850.

[28] C. Meyer, D. Robson, N. Rackovsky, V. Nadkarni, J. Gerich, Am. J. Physiol. Endocrinol. Metab. 273 (1997) 903–907.

[29] S.R. Morcos, Personal Communication, National Research Centre. El-Tahrir Street, Dokki, Cairo-Egypt, 1980.

[30] W.G. Nicola, T.H. Mikhail, R.B. Girgis, K.A. Ibrahim, Z.A. El-Khayat, D.F. Habib, Bull. Egypt. Soc. Physiol. Sci. 14 (4) (1994) 9–29.

[31] W.G. Nicola, T.H. Mikhail, R.B. Girgis, K.A. Ibrahim, Z. El Khayat, D.F. Habib, Med. J.Cairo. Univ. 64 (Suppl. 1) (1996) 111–125.

[32] M. Ozata, C. Oktenli, M. Gulec, T. Ozgurtas, F. Bulucu, K. Caglar, N. Bingol, A. Vural, C. Ozdemir, J. Clin. Endocrinol. Metab. 87 (2002) 853–858.

[33] D.L. Rainwater, J.L. Comuzziedeberg, M.C. Mahaney, J. Blangero, Atherosclerosis 132 (1997) 237–243.
[34] P. Sarraf, R.C. Frederich, E.M. Turner, et al., J. Exp. Med. 185 (1997) 171–175.
[35] M. Schroth, M. Gröschl, H.G. Dörr, W.F. Blum, W. Rascher, J. Dötsch, Eur. J. Endocrinol. 145 (2001) 463–468.
[36] K. Sharma, R.V. Considine, B. Michael, S.R. Dunn, L.S. Weisberg, B.R.C. Kurnik, P.B. Kurnik, J. O'connor, M. Sinha, J.F. Caro, Kidney Int. 51 (1997) 1980–1985.
[37] Steinberg, D., 1981. Metabolism of lipoproteins at the cellular level in relation to atherogenesis. in: lipoproteins atherosclerosis and coronary Heart disease vol. 1, No. 2, Elsevier North Holland, pp. 31-48.
[38] L. Tamer, B. Ercan, A. Unlu, N. Sucu, H. Recdemir, G. Eskandari, U. Atik, Indian Heart J. 54 (2002) 692–696.
[39] P. Trinder, Ann. Clin. Biochem. 6 (1969) 2427.
[40] M. Valle, F. Gascon, R. Martos, F. Bermudo, P. Ceballos, A. Suanes, Int. J. Obes. 27 (2003) 13–18.
[41] N.D. Vaziri, Kidney Int. 63 (2003) 1964–1976.
[42] A.W. Wahlefeld, In Methods of Enzymatic Analysis, vol. 5, in: H.U. Bergmeyer (Ed.), Academic Press, New York, 1974, pp. 1831–1835.
[43] A. Wasilewska, B. TomasZewska, W. Zoch-zwierz, A. Biernacka, Pediatr. Nephrol. 20 (2005) 597–602.
[44] D.M. Wu, M.H. Shen, N.F. Chu, Eur. J. Epidemiol. 17 (2001) 911–916.
[45] J. Zeng, B.W. Patterson, K. Samuel, R. Martin Dl, S. Dagogo-Jack, W.M. Kohrt, S.B. Miller, M. Landt, Am. J. Physiol. Endocrinol. Metab. 273 (1997) 1102–1106.
[46] Y. Zhang, R. Proenca, M. Maffei, et al., Nature 372 (1994) 425–432.